

§Appl. No. 09/869,595
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Reply to Office Action of, December 17, 2004

Listing of Claims:

Please amend the claims as follows:

Claim 1 (Currently Amended) A fusion protein comprising at least three domains, where

- a first domain mediates a membrane localization of the fusion protein in a cellular context, wherein said membrane localization signal comprises an amino acid sequence of a farnesylation signal or prenylation signal and
- a second domain has or presumably has a ligand-binding function of a nuclear receptor, which comprises an amino acid sequence of the receptor portion of a steroid receptor,
- a third domain has an activity which comprises an amino sequence of a naturally occurring Ras protein that is able to activate a signal pathway connected to a Ras protein in a cell, characterized in that when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain, the third domain cannot exert its activity to activate a signal pathway connected to a Ras protein in a cell, despite membrane localization.

Claim 2 (Original) A fusion protein as claimed in claim 1, characterized in that the individual domains are arranged within the fusion protein in the direction from the N terminus to the C terminus in the sequence first domain, second domain, third domain or in the sequence third domain, second domain, first domain.

Claim 3 (Previously Presented) A fusion protein as claimed in claim 1, characterized in that the inability of the third domain to activate a signal pathway connected to a Ras protein when there is a lack of binding of ligand to the second domain derives from the fact that when there is a lack of binding of ligand to the second domain the third domain can be complexed by a multiprotein complex attaching to the fusion protein in such a way that the third domain is unable

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to exert its activity to activate a signal pathway connected to a Ras protein in a cell.

Claim 4 (Original) A fusion protein as claimed in claim 3, characterized in that it comprises an additional protein section within the second domain or as fourth domain for attachment of the multiprotein complex.

Claim 5 (Cancelled)

Claim 6 (Cancelled)

Claim 7 (Cancelled)

Claim 8 (Currently Amended) A fusion protein as claimed in claim 1 6, characterized in that the amino acid sequence of the second domain comprises an amino acid sequence which is derived from the amino acid sequence of a receptor section of a naturally occurring nuclear steroid receptor by mutation, in particular by attachment, substitution, deletion, insertion and/or modification of one or more amino acids or groups of amino acids.

Claim 9 (Cancelled)

Claim 10 (Currently Amended) A fusion protein as claimed in claim 1, characterized in that the third domain has the activity of ~~an active and, in particular, a~~ constitutively active Ras protein.

Claim 11 (Previously Presented) A fusion protein as claimed in claim 1, characterized in that the third domain has the activity of a functional guanine nucleotide exchange factor.

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Claim 12 (Original) A fusion protein as claimed in claim 11, characterized in that the amino acid sequence of the third domain is derived from the amino acid sequence of the CDC25 protein from *Saccharomyces cerevisiae*, of an SOS protein from a mammal or of an SOS-like protein from any organism.

Claim 13 (Original) A fusion protein as claimed in claim 12, characterized in that the amino acid sequence of the third domain comprises at least the amino acid sequence sections of the CDC25 protein, of the SOS protein or of the SOS-like protein which are necessary for the activity of one of these proteins.

Claim 14 (Currently Amended) A fusion protein as claimed in claim 1, characterized in that the third domain comprises an amino acid sequence which is derived from the amino acid sequence of a naturally occurring Ras protein ~~or of a naturally occurring guanine nucleotide exchange factor or of the sections thereof necessary for the activity by mutation, and in particular~~ by attachment, substitution, deletion, insertion and/or modification of one or more amino acids or groups of amino acids.

Claim 15 (Previously Presented) A DNA molecule which encodes the fusion protein as claimed in claim 1.

Claim 16 (Original) A vector, in particular plasmid, cosmid, viral or phage genome, comprising at least one DNA molecule as claimed in claim 15.

Claim 17 (Original) A vector as claim in claim 16, which is suitable for the transformation or transfection of a host cell.

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Claim 18 (Previously Presented) A vector as claimed in claim 16, which is suitable for expression of at least one fusion protein, characterized in that it comprises at least one DNA molecule as claimed in claim 16 under the control of one or more promoters capable of functioning in a host cell.

Claim 19 (Previously Presented) A cell comprising a fusion protein as claimed in claim 1, characterized in that when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain of the fusion protein the third domain is unable to exert its activity to activate a signal pathway connected to a Ras protein in the cell, despite membrane localization, but when there is binding of ligand to the second domain or, in the alternative variant, when the ligand dissociates off from the second domain a conformational change is brought about with effects on the third domain so that the third domain is able to exert its activity to activate a signal pathway connected to a Ras protein in the cell.

Claim 20 (Original) A cell as claimed in claim 19, characterized in that the inability of the third domain of the fusion protein to activate a signal pathway connected to a Ras protein where there is a lack of binding of ligand to the second domain of the fusion protein derives from the fact that when there is a lack of binding of ligand to the second domain the third domain can be complexed by a multiprotein complex attaching to the fusion protein in such a way that the third domain is unable to exert its activity to activate a signal pathway connected to a Ras protein in the cell, but when there is binding of ligand to the second domain a conformational change is brought about with effects on the third domain, resulting in the multiprotein complex at least partly dissociating off from the fusion protein, and the third domain being able to exert its activity to activate a signal pathway connected to a Ras protein in the cell.

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Claim 21 (Currently Amended) A cell as claimed in claim 19, characterized in that it comprises two or more said fusion proteins ~~as claimed in claim 19~~.

Claim 22 (Currently Amended) A cell as claimed in claim 19, characterized in that the cell is a single-cell prokaryotic or eukaryotic cell ~~and, in particular, a yeast cell, specifically a yeast cell lacking cell walls~~.

Claim 23 (Currently Amended) A cell as claimed in claim 19, characterized in that ~~in the absence of fusion protein at least under certain conditions a the intrinsic~~ signal pathway connected to a Ras protein ~~is inactivated cannot be activated~~ in the cell.

Claim 24 (Original) A cell as claimed in claim 23, characterized in that it comprises at least one fusion protein with a third domain which is able to activate the signal pathway connected to a Ras protein in the cell, which is inactive or inactivatable in the absence of the fusion protein.

Claim 25 (Previously Presented) A cell as claimed in claim 23, characterized in that the signal pathway connected to a Ras protein acts on the cell cycle and its activation is essential for cell reproduction or the signal pathway connected to a Ras protein alternatively serves to activate transcription factors for genes which are not essential for cell reproduction.

Claim 26 (Previously Presented) A cell as claimed in claim 23, characterized in that the activability of the signal pathway connected to a Ras protein is temperature-dependent in the absence of fusion protein.

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Claim 27 (Original) A cell as claimed in claim 26, characterized in that the lack of activatability of the signal pathway connected to Ras protein in the absence of fusion protein at particular temperatures is derived from at least one mutation of a guanine nucleotide exchange factor intrinsic to the cell, which has the effect that the latter is incapable of functioning above a particular temperature.

Claim 28 (Original) A cell as claimed in claim 27, characterized in that it is a cell of the *Saccharomyces cerevisiae* yeast strain cdc25-2 or is derived from the latter.

Claim 29 (Previously Presented) A cell as claimed in claim 27, characterized in that the cell comprises a fusion protein whose third domain has the activity of a functional guanine nucleotide exchange factor.

Claim 30 (Currently Amended) A cell as claimed in claim 27, characterized in that the cell comprises a fusion protein whose third domain has the activity of ~~an active and, in particular,~~ a constitutively active Ras protein.

Claim 31 (Previously Presented) A cell as claimed in claim 26, characterized in that the lack of activatability of the signal pathway subsequent to a Ras protein in the absence of fusion protein at particular temperatures is derived from at least one mutation of a Ras protein intrinsic to the cell, which has the effect that the latter is incapable of functioning above a particular temperature.

Claim 32 (Original) A cell as claimed in claim 23, characterized in that the lack of activatability of the signal pathway connected to a Ras protein in the absence of fusion protein derives from a deletion of the membrane-localization signal, in particular farnesylation signal, of

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the Ras protein intrinsic to the cell or from a mutation of this membrane-localization signal which has the effect that the Ras protein no longer binds to cellular membranes.

Claim 33 (Currently Amended) A cell as claimed in claim 31, characterized in that the cell comprises a fusion protein whose third domain has the activity of ~~an active and, in particular, a constitutively active Ras protein.~~

Claim 34 (Previously Presented) A cell as claimed in claim 19, characterized in that it is applied to a solid carrier.

Claim 35 (Original) A cell as claimed in claim 34, characterized in that it is immobilized on biochips.

Claim 36 (Previously Presented) An *in vivo* assay for determining the suitability of a test substance as ligand for a receptor section of a nuclear receptor, characterized by the following steps:

(a) contacting the test substance with cells as claimed in claim 23 under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, where the fusion protein present in the cells contains a second domain comprising said receptor section, and a third domain which, when there is binding of ligand to the second domain, is able to activate the inactive signal pathway connected to a Ras protein,

(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place, where detection of the activation of the signal pathway connected to a Ras protein indicates the ability of the test substance to bind to the second domain of the fusion protein and thus to the receptor section.

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Claim 37 (Original) An assay as claimed in claim 36, where step (b) comprises detecting the activation of the signal pathway connected to Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, where detection of the expression of the reporter gene indicated the ability of the test substance to bind to the second domain of the fusion protein and, accordingly, to the receptor section.

Claim 38 (Original) An assay as claimed in claim 36, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the ability of the test substance to bind to the second domain of the fusion protein and, accordingly, to the receptor section.

Claim 39 (Previously Presented) An *in vivo* assay for determining the suitability of a test substance as ligand for a receptor section of a nuclear receptor, characterized by the following steps:

(a) contacting the test substance with cells as claimed in claim 23 under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, where the fusion protein present in the cells contains a second domain comprising said receptor section, and a third domain which, only when there is binding of ligand to the second domain, is able to activate the inactive signal pathway connected to a Ras protein,

(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place,

(c) investigating cells employed in step (a) under conditions with which the signal

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pathway connected to a Ras protein in the cells cannot be activated in the absence of the fusion protein, for activation of the signal pathway connected to a Ras protein in the absence of the test substance, where detection of the activation of the signal pathway connected to a Ras protein in the absence of the test substance and the inactivity of the signal pathway connected to a Ras protein in the presence of the test substance indicates the ability of the test substance to bind to the second domain of the fusion protein and thus to the receptor section.

Claim 40 (Previously Presented) An assay as claimed in claim 36, characterized in that the test substance is a naturally occurring substance and, in particular, a hormone, in particular a steroid hormone, a vitamin, thyroxine or retinoic acid.

Claim 41 (Previously Presented) An assay as claimed in claim 36, characterized in that the test substance is a non-naturally occurring substance.

Claim 42 (Currently Amended) An assay as claimed in claim 41, characterized in that the test substance is ~~a synthetic derivative of a natural ligand or a poison, in particular dioxin.~~

Claim 43 (Previously Presented) A screening method for unknown ligands of a particular nuclear receptor, characterized in that an assay method as claimed in claim 36 is employed for the screening.

Claim 44 (Previously Presented) An *in vivo* assay for detecting the presence of a ligand for a receptor section of a nuclear receptor in a sample which possibly contains the latter, characterized by the following steps:

(a) contacting the sample with cells as claimed in claim 23 under conditions with which a signal pathway connected to a Ras protein in the cell cannot be activated in the absence

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of the fusion protein, where the fusion protein comprises a second domain comprising said receptor section, and a third domain which is able to activate the signal pathway connected to a Ras protein in the cells,

(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place, where detection of the activation of the signal pathway connected to a Ras protein indicates the presence of a ligand for the second domain of the fusion protein and thus for the receptor section of a nuclear receptor in the sample.

Claim 45 (Original) An assay as claimed in claim 44, where step (b) comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, where detection of the expression of the reporter gene indicates the presence of a ligand for the second domain of the fusion protein and, accordingly, for the receptor section of a nuclear receptor in the sample.

Claim 46 (Original) An assay as claimed in claim 44, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the presence of a ligand for the second domain of the fusion protein and, accordingly, for the receptor section of a nuclear receptor in the sample.

Claim 47 (Previously Presented) An *in vivo* assay for detecting the presence of a ligand for a receptor section of a nuclear receptor in a sample which possibly contains the latter, characterized by the following steps:

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- (a) contacting the sample with cells as claimed in claim 23 under conditions with which the signal pathway connected to a Ras protein in the cell cannot be activated in the absence of fusion protein, where the fusion protein comprises a second domain comprising said receptor section, and a third domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells,
- (b) investigating whether an activation of the signal pathway connected to a Ras protein has taken place,
- (c) investigating cells employed in step (a) under conditions with which a signal pathway connected to a Ras protein in the cells cannot be activated in the absence of fusion protein, for activation of the signal pathway connected to a Ras protein in the absence of the sample, where a detection of the activation of the signal pathway connected to a Ras protein in the absence of the sample and the inactivity of the signal pathway connected to a Ras protein in the presence of the sample indicates the presence of a ligand for the second domain of the fusion protein and thus for the receptor section of a nuclear receptor in the sample.

Claim 48 (Previously Presented) A screening method for unknown ligands of a particular nuclear receptor in a sample, characterized in that an assay method as claimed in claim 44 is employed for the screening.

Claim 49 (Previously Presented) An *in vivo* assay for the quantitative determination of the concentration of a ligand for the receptor section of a nuclear receptor in a sample which contains the latter, characterized by the following steps:

- (a) contacting an aliquot of the sample with cells as claimed in claim 23 under conditions with which a signal pathway connected to a Ras protein in the cell cannot be activated in the absence of the fusion protein, where the fusion protein present in the cells comprises said receptor section, and contains a second domain comprising said receptor section, and a third

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domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells,

- (b) detecting quantitatively the extent of the activation of the signal pathway connected to a Ras protein by direct or indirect means,
- (c) measuring the concentration of the ligand in the sample by comparing the measured extent of activation with corresponding values measured for known standard concentrations of the ligand.

Claim 50 (Original) An assay as claimed in claim 49, characterized in that the quantitative detection of the extent of activation of the signal pathway connected to a Ras protein in step (b) takes place indirectly by determining the amount present in the cells of a transcription or translation product of a reporter gene whose expression takes place only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, at a particular time or the expression rate of this reporter gene based on the transcription or translation product under said conditions, and in step (c) the measurement of the concentration of the ligand in the sample takes place by comparing the measured values with corresponding values measured for known standard concentrations of the ligand.

Claim 51 (Original) An assay as claimed in claim 49, characterized in that in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction are employed, and the quantitative detection of the extent of the activation of the signal pathway connected to a Ras protein in step (b) takes place indirectly by determining the reproduction of the cells at a fixed time or the reproduction rate of the cells under said conditions, and in step (c) the measurement of the concentration of the ligand in the samples takes place by comparing the measured values with corresponding values measured for known standard concentrations of the ligand.

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Claim 52 (Previously Presented) An *in vivo* assay for detecting whether a compound is able to alter a binding activity of a receptor section of a nuclear receptor in relation to a ligand, characterized by the following steps:

- (a) contacting the ligand in the presence of the compound with cells as claimed in claim 23 under conditions with which the compound can diffuse into the cells or it is produced by the cells, and with which in the absence of fusion protein a signal pathway connected to a Ras protein in the cells cannot be activated, where the fusion protein present in the cells comprises a second domain comprising said receptor section, and a third domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells only when there is binding of the ligand or, alternatively, only when there is lack of binding of ligand to the second domain,
- (b) investigating whether and, where appropriate, to what extent activation of the signal pathway connected to a Ras protein takes place,
- (c) comparing the result of the investigation in step (b) with a result of an investigation obtained when the assay is carried out in the absence of the compound.

Claim 53 (Original) An assay as claimed in claim 52, characterized in that step (b) comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, and the quantitative detection, which takes place where appropriate, of the extent of the activation of the signal pathway connected to a Ras protein comprises determining the amount, present in the cells, of transcription or translation product of the reporter gene at a particular time or the expression rate of this reporter gene based on the transcription or translation product under said conditions, and in the case where the comparison in step (c) reveals that stronger expression of the reporter gene occurs in the presence of the compound, an agonistic effect of the compound is indicated, and in the case where the comparison in (c) reveals

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that lower expression of the reporter gene occurs in the presence of the compound, an antagonistic effect of the compound is indicated.

Claim 54 (Original) An assay as claimed in claim 53, characterized in that it is carried out under conditions with which no reproduction of the cells occurs.

Claim 55 (Original) An assay as claimed in claim 52, where in step (a) there is use of cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction, and step (b) comprises investigating whether and, where appropriate to what extent, the cells are able to reproduce under said conditions, and in the case where the comparison in step (c) reveals that greater cell reproduction occurs in the presence of the compound, an agonistic effect of the compound is indicated, and in the case where the comparison in step (c) reveals that less cell reproduction occurs in the presence of the compound, an antagonistic effect of the compound is indicated.

Claim 56 (Previously Presented) An *in vivo* assay for detecting whether a polypeptide or protein has a ligand-binding function of a nuclear receptor, characterized by the following steps:

(a) contacting cells as claimed in claim 23 with the ligand under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, where the fusion protein present in the cells comprises a second domain which comprises the polypeptide or protein to be investigated, and a third domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells when there is binding of ligand to the second domain,

(b) investigating whether an activation of the signal pathway connected to a Ras protein has taken place, where detection of the activation of the signal pathway connected to a Ras protein indicates that the second domain of the fusion protein and, accordingly, the

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polypeptide or protein to be investigated has a ligand-binding function of a nuclear receptor.

Claim 57 (Original) An assay method as claimed in claim 56, characterized in that the fusion protein present in the cells comprises a second domain which contains a receptor section derived from a naturally occurring receptor section by mutation.

Claim 58 (Previously Presented) An assay as claimed in claim 56, where step (b) comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, where detection of the expression of the reporter gene indicates the presence of a ligand-binding function of the second domain of the fusion protein and, accordingly, of the polypeptide or protein to be investigated.

Claim 59 (Previously Presented) An assay as claimed in claim 56, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the presence of a ligand-binding function of the second domain of the fusion protein and, accordingly, of the polypeptide or protein to be investigated.

Claim 60 (Previously Presented) An *in vivo* assay for detecting whether a polypeptide or protein has a ligand-binding function of a nuclear receptor, characterized by the following steps:
(a) contacting cells as claimed in claim 23 with the ligand under conditions with which a signal pathway connected to a Ras protein in the cells cannot be activated in the absence

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of the fusion protein, where the fusion protein present in the cells comprises a second domain which comprises the polypeptide or protein to be investigated, and a third domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells only when there is a lack of binding of ligand to the second domain,

- (b) investigating whether an activation of the signal pathway connected to a Ras protein has taken place,
- (c) investigating cells as employed in step (a) under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, for activation of the signal pathway connected to a Ras protein in the absence of ligand, where a detection of the activation of the signal pathway connected to a Ras protein in the absence of the ligand and the inactivity of the signal pathway connected to a Ras protein in the presence of the ligand indicates that the second domain of the fusion protein and, accordingly, the polypeptide or protein to be investigated has a nuclear receptor.

Claim 61 (Previously Presented) A kit for use in an assay or screening method as claimed in claim 36, characterized in that it comprises cells as claimed in claim 36.

Claim 62 (Previously Presented) A kit for use in an assay as claimed in claim 36, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- b) one or more transformation or transfection vectors which contain at least one DNA sequence which encodes a fusion protein as claimed in claim 36, where the fusion protein comprises a third domain which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is lack of binding, or alternatively, when there is binding of ligand to the second domain,

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- c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal pathway connected to a Ras protein in these cells.

Claim 63 (Previously Presented) A kit for use in an assay as claimed in claim 36, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- b) a transformation or transfection vector which has, in suitable arrangement,
 - a DNA sequence which encodes a first domain of a fusion protein as defined in claim 36,
 - a DNA sequence which encodes a third domain of a fusion protein as defined in claim 36 and which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain, and
 - a suitably arranged insertion site for functional insertion of a DNA sequence which encodes a second domain as defined in claim 36,

where, after insertion of a DNA sequence for the second domain, the vector comprises a complete gene for a fusion protein as claimed in claim 36,

- c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal transduction pathway connected to a Ras protein in these cells.

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Claim 64 (Previously Presented) A kit for use in an assay as claimed in claim 56, characterized in that it comprises cells as claimed in claim 56, where the fusion protein present therein comprises a second domain comprising a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor.

Claim 65 (Previously Presented) A kit for use in an assay as claimed in claim 56, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- b) one or more transformation or transfection vectors which comprise at least one DNA sequence which encodes a fusion protein as claimed in claim 56, whose second domain comprises a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor, and whose third domain is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain,
- c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal transduction pathway connected to a Ras protein in these cells.

Claim 66 (Previously Presented) A kit for use in an assay as claimed in claim 56, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- b) a transformation or transfection vector which has, in suitable arrangement,
 - a DNA sequence which encodes a first domain of a fusion protein as defined in

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claim 56, and

- a DNA sequence which encodes a third domain of a fusion protein as defined in claim 56 and which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain, and
- a suitably arranged insertion site for functional insertion of a DNA sequence which encodes a second domain comprising a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor,

where, after insertion of a DNA sequence for the second domain, the vector comprises a complete gene for a fusion protein as claimed in claim 56, where the second domain comprises a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor,

- c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal pathway connected to a Ras protein in these cells.

Claim 67 (Previously Presented) A kit as claimed in claim 61, in which the cells additionally contain a construct comprising a binding site for a transcription factor whose activation results from an activation of a specific ras signal pathway whose activation is to be detected by the assay, a minimal promoter and a reporter gene functionally linked thereto, where the minimal promoter is activated as a result of binding of the activated transcription factor to its binding site.

Claim 68 (Previously Presented) A kit as claimed in claim 61, characterized in that it additionally contains a transformation or transfection vector with a construct comprising a binding site for a transcription factor whose activation results from an activation of a specific ras

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signal pathway whose activation is to be detected by the assay, a minimal promoter and a reporter gene functionally linked thereto, where the minimal promoter is activated as a result of a binding of the activated transcription factor to its binding site.

Claim 69 (Previously Presented) A kit as claimed in claim 61, characterized in that it additionally contains a transformation or transfection vector with a construct comprising a binding site for a transcription factor whose activation results from an activation of a specific ras signal pathway whose activation is to be detected by the assay, a minimal promoter and an insertion site, suitably arranged for expression controlled by the minimal promoter, for insertion of a gene for a reporter protein, where the minimal promoter is activated as a result of a binding of the activated transcription factor to its binding site.

Claim 70 (Previously Presented) A kit as claimed in claim 61, which contains the cells immobilized on a solid carrier, in particular on microtiter plates or biochips.

Claim 71 (Previously Presented) A method for identifying polypeptides or proteins, in particular receptors, which have a ligand-binding function of a receptor, which comprises:

- preparing a cell as claimed in claim 1 with a fusion protein having the features described in claim 1 and comprising the whole of such a polypeptide or protein or a part of such a polypeptide or protein which presumably contains the sequence sections essential for the ligand-binding function, and
- using this cell to carry out an *in vivo* assay method for detecting whether a polypeptide or protein has a ligand-binding function of a receptor, as claimed in claim 1.

Claim 72 (Currently Amended) A method for identifying a ligand for a binding section of a steroid receptor, a compound able to alter the binding activity of a ligand-binding section of a

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steroid receptor in relation to a ligand, or a polypeptide or protein having a ligand-binding function of a steroid receptor, where a presumed ligand, a presumed modifying compound or a polypeptide or protein with presumed ligand-binding function, which have ~~respectively~~ been obtained by derivatizing ~~derivatization~~ one or more times ~~starting from~~ a ligand, modifying compound, polypeptide or protein identified by means of the assay, ~~screening or identification~~ methods as claimed in claim 36, is subjected to one of the ~~assay, screening or identification~~ methods as claimed in claim 36.

Claim 73 (Cancelled)

Claim 74 (Original) A method for identifying polypeptides or proteins, in particular receptors, which has ligand-binding function of a receptor, which comprises:

- preparing a cell as claimed in claim 1 with a fusion protein having the features described in claim 1 and comprising the whole of such a polypeptide or protein or a part of such a polypeptide or protein which presumably contains the sequence sections essential for the ligand-binding function, and
- using this cell to carry out an *in vitro* assay method for detecting whether a polypeptide or protein has a ligand-binding function of a receptor, as claimed in any of claims 56 to 60.

Claim 75 (Original) The use of ligand, of a compound and/or of a polypeptide or protein as claimed in claim 71 as lead substance for developing ligands, compounds and polypeptides or proteins derived therefrom.

Claim 76 (Original) A method for preparing a ligand for a binding section of a receptor, a compound which is able to alter a binding activity of a ligand-binding section of a receptor in relation to a ligand, or a polypeptide or protein which has a ligand-binding function of a receptor,

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by derivatization one or more times starting from a ligand, modifying compound, polypeptide or protein identified by the assay, identification, screening or preparation methods as claimed in any of claims 36 to 48, 52 to 60 and 74.

Claim 77 (Original) A nucleic acid molecule obtained starting from a polypeptide or peptide, in particular receptor, identified or prepared by the assay or preparation methods as claimed in any of claims 56 to 60, 74 and 76, by a method which comprises the provision of a gene encoding the polypeptide or protein, or a part, which comprises at least the nucleic acid sequence sections essential for the activity of the encoded polypeptide or protein, in essentially pure form.

Claim 78 (Original) The use of a nucleic acid molecule as claimed in claim 77 for preparing a gene therapeutic agent.

Claim 79 (New) A cell as claimed in claim 22 which is a yeast cell.

Claim 80 (New) A cell as claimed in claim 79 which is a yeast cell lacking cell walls.